

A New Species of *Strobilanthes* (Acanthaceae) from Lanyu (Orchid Island), Taiwan, with Special Reference to the Flower Structure

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A new species, *Strobilanthes lanyuensis* Seok, C. F. Hsieh & J. Murata, is described from Lanyu (Orchid Island), Taiwan. The chloroplast DNA *trnL* (UAA) 5'-exon–*trnF* (GAA) intergenic spacer sequence data places this species in the *Parachampionella* group. Within the group *S. lanyuensis* is distinct in having a white corolla with the filament curtain above the corolla tube, so that the staminal deployment is from above the pollinator. A survey of herbarium specimens indicated that all specimens of *Strobilanthes* so far collected from Lanyu should in fact be ascribed to *Strobilanthes lanyuensis*.

Key words: Resupination, *Strobilanthes lanyuensis*, systematics, Taiwan.

Strobilanthes Blume (Acanthaceae) is a large, variable genus. Estimates of the number of species in *Strobilanthes* s. l. range from 350 (Mabberley 1987) to more than 450 (Terao 1983) and the generic circumscription and species delimitations remain ambiguous. *Strobilanthes* s. l. is distributed throughout southern and southeastern Asia, with the northeastern margin of the range being Taiwan, Japan and Korea, where *S. flexicaulis* Hayata and its putative allies (here called the *Parachampionella* group) constitute one of the most taxonomically difficult groups in the area. The genus *Parachampionella* was established by Bremekamp (1944) to accommodate a small group of species with globose, echinulate pollen and three-parted calyx (i.e., three of the five calyx lobes are partly fused to each other) originally described under *Strobilanthes*, i. e., *Parachampionella tashiroi* (Hayata) Bremek. from the Ryukyu Islands

and *P. rankanensis* (Hayata) Bremek. from Taiwan. Although Bremekamp (1944) described the corolla as being not resupinate, that statement may be incorrect. Later, Hsieh and Huang (1978) added a third species, *Parachampionella flexicaulis* (Hayata) C. F. Hsieh & T. C. Huang, to the genus. *Strobilanthes flexicaulis* Hayata (1915) was first described as a Taiwanese endemic species, but then some of the Ryukyu plants were included in this species (Masamune 1955, Walker 1976). Hatusima (1956) separated the Ryukyu element from *S. flexicaulis* as *S. glandulifera* Hatusi. After the taxonomic consideration in Yamazaki (1991), he reduced *S. glandulifera* and *S. tashiroi* to synonymy with *S. flexicaulis* (Yamazaki 1993). In the latest treatment of the so-called two-lipped species of *Strobilanthes* s. l., which share the distinctive upper calyx lip with three fused lobes, Wood and Scotland (2003) recognized *S. tashiroi*, *S. rankanensis*, *S.*

flexicaulis (including *S. glandulifera* as a synonym), and *S. perplexa* J. R. I. Wood, a new species from Sumatra, under *Parachampionella*. In their treatment, however, the authors clearly distinguished species with globose and echinulate pollen from species with ellipsoid and scalariform pollen so that *S. tashiroi* is recognized as distinct from *S. flexicaulis*.

During recent field research as apart of our study of *Strobilanthes* in Japan and Taiwan, we collected some unknown plants, here named *Strobilanthes lanyuensis*, from Lanyu (Orchid Island) off Taiwan's south-east coast. We initially attributed them to *S. flexicaulis* Hayata (e. g., Hsieh and Huang 1978, 1998), but preliminary assessment of morphological characters showed that they differed from *S. flexicaulis* in floral characters. To determine the systematic position and circumscription of *S. lanyuensis*, a molecular phylogenetic analysis and comparison of the morphology and pollen were carried out.

Materials and Methods

Field collections — Field collections and observations of the Taiwanese species of *Strobilanthes* were made on mainland Taiwan in April 2001 and on Lanyu Is. in March 2002. The Japanese species of the *Parachampionella* group were observed during six field expeditions to the Ryukyu Islands during 2000 and 2003. Dried herbarium specimens of our collections have been deposited in the Herbarium, Botanical Gardens, The University of Tokyo (TI), and duplicates of some of them in the Herbarium of National Taiwan University (TAI). We also collected flower specimens preserved in FAA for morphological observations and leaf samples dried with silica gel for DNA analysis.

Molecular phylogenetic analysis — To determine the phylogenetic position of *S. lanyuensis*, molecular phylogenetic analysis

was carried out for the six taxa of the *Parachampionella* group in Japan and Taiwan and additional species of *Strobilanthes*. *Strobilanthes cernua* Blume, the type species of the genus, was examined for the first time. *Ruellia* was selected as the outgroup and *Hemigraphis* was included as a putative sister group based on the molecular phylogeny of the Acanthaceae (Scotland et al. 1995). Sequences data from *Ruellia californica* from GenBank was also included (Table 1). Genomic DNA was extracted from leaves dried with silica gel using the CTAB (cetyltrimethylammonium bromide) method (Doyle and Doyle 1987). The nucleotide sequences of the chloroplast DNA *trnL* (UAA) 5'exon-*trnF* (GAA) intergenic spacer region (Taberlet et al. 1991) were determined by the standard PCR-direct sequencing method with ABI PRISM 377 DNA sequencer (Applied Biosystems, Foster City, California, USA). All sequences determined were deposited in the DNA Data Bank of Japan (DDBJ) (Table 1). Sequences were aligned using the CLUSTAL X program (Thompson 1997) with manual modifications. Phylogenetic analysis was performed by the neighbor-joining (NJ) method with Kimura-2-parameter using PAUP* ver. 4.0b10 (Swofford 2002). Bootstrap analyses were conducted using 1000 replicates.

Morphological observations — Dried herbarium specimens deposited in the Herbaria of University Museum of Kyoto University (KYO), National Taiwan University (TAI), the Botanical Gardens, The University of Tokyo (TI), and the National Museum of Natural Science, Taichung, Taiwan (TNM), plus living plant material and liquid preserved flowers obtained from the field and/or the Botanical Gardens, The University of Tokyo, were examined for all the Taiwanese and Japanese species. Observations were conducted using a Leica dissecting microscope and freehand illustrations were prepared. Flowers removed from herbarium

Table 1. List of *Strobilanthes* and allied genera examined for *trnL*–*trnF* sequence data

Taxon	Source and voucher	DDBJ accession number
<i>Strobilanthes anisophylla</i> T. Anders.	Cult. at BG, Setsunan Univ.	AB161981
<i>S. auriculata</i> Nees	Thailand, Chang Mai, Ban Sop Aep; Seok & al. 0112036 (TI)	AB161982
<i>S. cernua</i> Blume	Indonesia, West Java, Mt. Slamet; Imaichi s.n. (TI)	AB161980
<i>S. colorata</i> T. Anders.	Thailand, Chang Mai, Siriphon Waterfall; Seok & al. 0112040 (TI)	AB161983
<i>S. cusia</i> Kuntze	Cult. at BG, Univ. Tokyo	AB161984
<i>S. dyeriana</i> Hort. Sander.	Cult. at BG, Univ. Tokyo	AB161985
<i>S. echinata</i> Nees	Thailand, Chang Mai, Doi Inthanon, summit area; Seok & al. 0112055 (TI)	AB161986
<i>S. flexicaulis</i> Hayata	Taiwan, Kaohsiung, Taoyuan, Tengchi; Seok & al. 0104004 (TI)	AB161987
<i>S. formosana</i> S. Moore	Taiwan, Taipei, Mt. Tatun; Seok & al. 0104001 (TI)	AB161988
<i>S. glandulifera</i> Hatus.	Japan, Okinawa, Nago, Mt. Nagodake; Seok & al. 0102101 (TI)	AB161989
<i>S. gossypina</i> T. Anders.	Cult. at BG, Univ. Tokyo	AB161990
<i>S. hossei</i> C. B. Clarke	Thailand, Chang Mai, Chom Thong District.; Seok & al. 0112047 (TI)	AB161991
<i>S. japonica</i> Miq.	Cult. at BG, Univ. Tokyo	AB161992
<i>S. lanyuensis</i> Soek, C. F. Hsieh & J. Murata	Taiwan, Taitung, Lanyu island; Seok & Hsieh 0203002 (TI)	AB161993
<i>S. longespicata</i> Hayata	Taiwan, Pintung, Nanjenshan; Chao K. J. s. n. (TI)	AB161994
<i>S. oligantha</i> Miq.	Korea, Cheju, Kyore, Daechon-dong; Seok & al. 0008001 (TI)	AB161995
<i>S. pentstemonoides</i> T. Anders.	Thailand, Chang Mai, Wachirathan Waterfall; Seok & al. 0112036 (TI)	AB161996
<i>S. rankanensis</i> Hayata	Taiwan, Nantou, Mt. Hohuan; Seok & al. 0104009 (TI)	AB161997
<i>S. repanda</i> (Blume) J. R. Bennett	Thailand, Nakhon Nayok, Haew Suwat Waterfall; Seok & al. 0112003 (TI)	AB161998
<i>S. serrata</i> Imlay	Thailand, Chang Mai, Siriphon Waterfall; Seok & al. 0212041 (TI)	AB161999
<i>S. tashiroi</i> Hayata	Japan, Okinawa, Nago, Mt. Nagodake; Seok & al. 0102201 (TI)	AB162000
<i>S. wakasana</i> T. Wakasugi & N. Naruhashi	Cult. at BG, Univ. Tokyo	AB162001
<i>Hemigraphis cumingiana</i> Villar	Taiwan, Taitung, Lanyu island; Seok and Hsieh 0203021 (TI)	AB162024
<i>H. glaucescens</i> C. B. Clarke	Thailand, Nakhon Nayok, Khao Yai N.P.; Seok & al. 0212013 (TI)	AB162026
<i>H. okamotoi</i> Masam.	Cult. at BG, Univ. Tokyo	AB162022
<i>H. quadrifaria</i> T. Anders.	Thailand, Chang Mai, Mae Fang N.P.; Seok & al. 0212032 (TI)	AB162027
<i>H. reptans</i> Engl.	Taiwan, Taitung, Lanyu island; Seok and Hsieh 0203020 (TI)	AB162023
<i>Ruellia graeziana</i> Backer	Cult. at BG, Univ. Tokyo	AB162025
<i>R. californica</i> I. M. Johnston.	(Data obtained from GenBank)	AF063115

specimens were dehydrated prior to dissection, and were cut longitudinally with microscissors along the central vascular trace of the lower lip. Pollen grains were removed from dried herbarium specimens, acetolyzed according to standard technique (Erdtman 1960), air dried onto stubs from 99 % ethanol, sputter coated with gold and observed with a JEOL JSM 820 SEM.

Result and Discussion

Molecular phylogeny

The molecular phylogenetic tree constructed by the NJ-method based on the sequence variation of *trnL* (UAA) 5' exon–*trnF*

(GAA) intergenic spacer region (725 bases) is shown in Fig. 1, which shows the monophyly of the *Parachampionella* group, although bootstrap probability is not high (63 %). Indeed, all the species of the *Parachampionella* group examined, excepting *S. flexicaulis*, have the same sequence; *S. flexicaulis* varies only in a single base from the others. It is evident that *S. lanyuensis* is phylogenetically comparable to the other members of the *Parachampionella* group.

Morphological observations

Pollen: The pollen of *S. lanyuensis* was observed for the first time together with *S.*

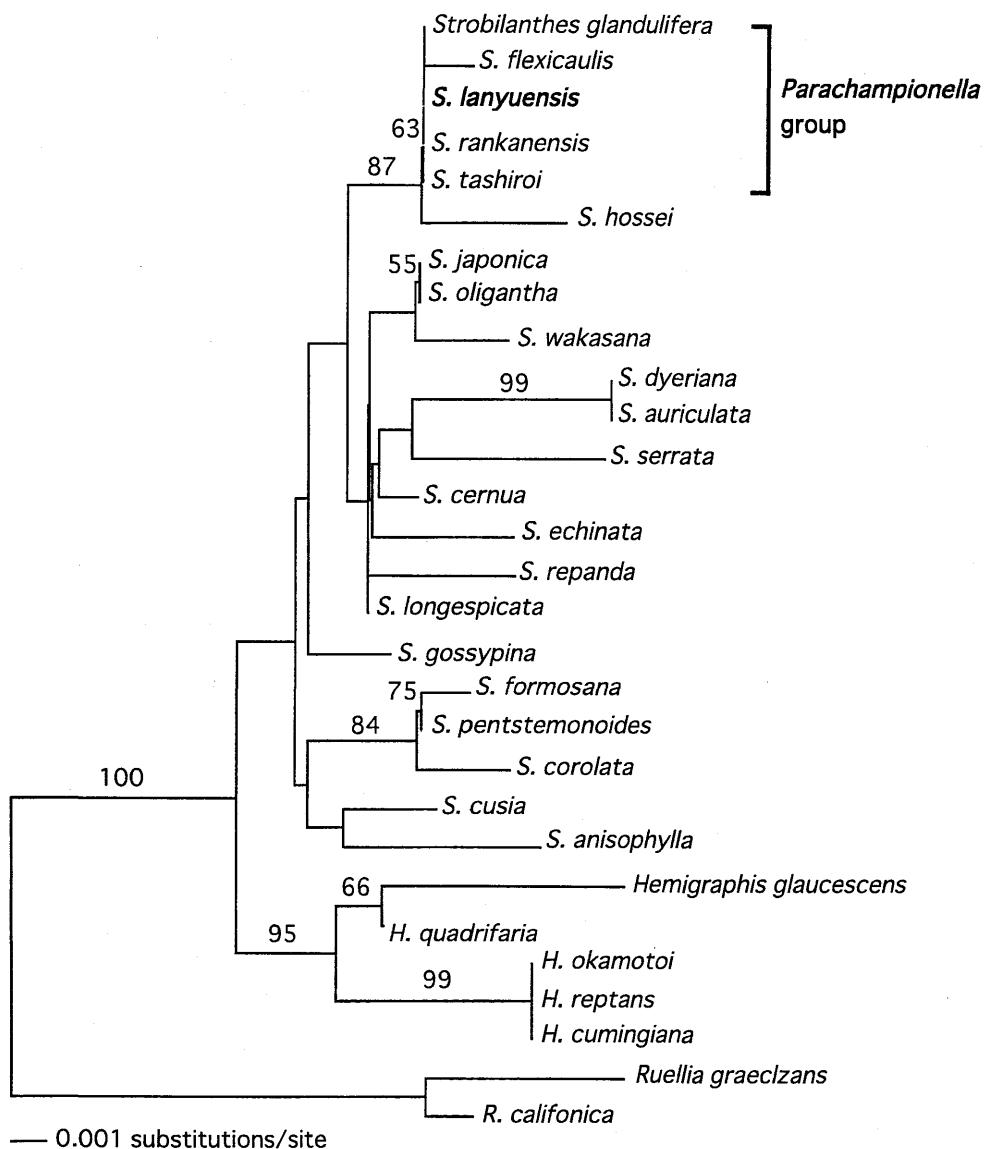


Fig. 1. Neighbour-joining tree for species of *Strobilanthesinae* based on 725 bp of *trnL* (UAA) 5' exon-*trnF* (GAA) intergenic spacer region sequences. Numbers above branches show bootstrap values (1000 replicates) where branch support is more than 50 %.

flexicaulis for comparison using SEM (Fig. 2). The pollen type of the two species is correspondent to the Type 3 of Carine and Scotland (1998). Pollen grains are ellipsoid, tricolporate and have pseudocolpi. The ribs between pseudocolpi are ornamented with a

coarse ladder-like reticulum, whose lumina are perforate. In *S. lanyuensis* the pollen has 14 longitudinal ribs, prolate and P : E ratio = 1.63 (64–66 μ m \times 40–41 μ m). In *S. flexicaulis*, the pollen has 14–15 longitudinal ribs, prolate and P : E ratio = 1.47 (58–68 μ m \times

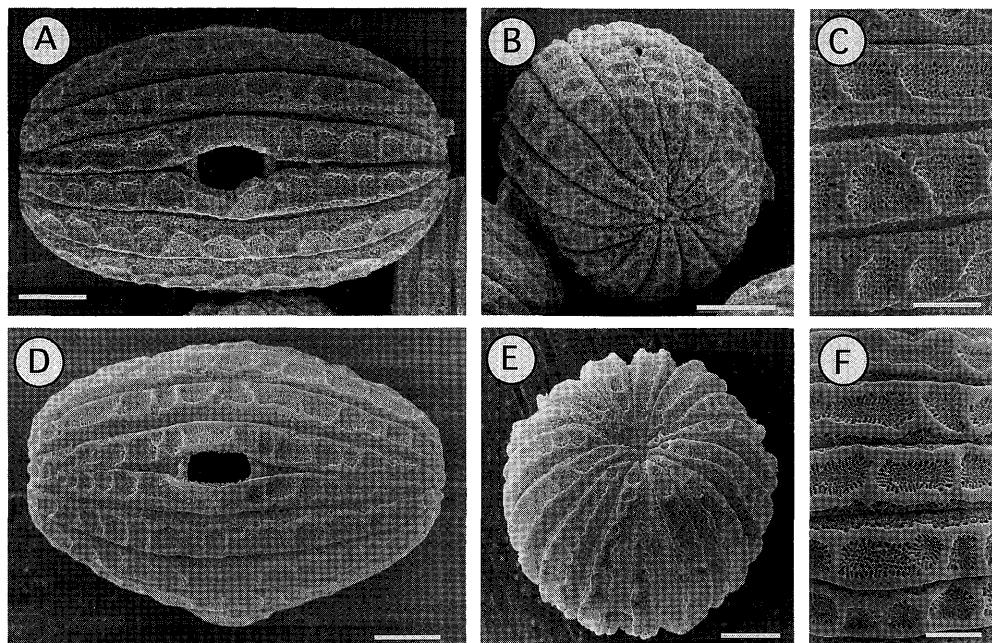


Fig. 2. *Strobilanthes* pollen grains (SEM). A-C: *S. lanyuensis* (Seok & Hsieh 0203007). D-F: *S. flexicaulis* (Seok & al. 0104006). Scale bars: A, B, D, E = 10 μ m; C, F = 5 μ m.

34–46 μ m). This type of ellipsoid pollen with scalariform sculpturing of *S. lanyuensis* and *S. flexicaulis* is distinct from the echinate, globose one of *S. tashiroi*, *S. rankanensis* and *S. perplexa* (Fig. 13 in Wood and Scotland 2003).

Flower structure: The inflorescence of the *Parachampionella* group is a raceme with each flower subtended by a bract (Fig. 3). The short pedicel bears a pair of bracteoles. Three of the five calyx lobes, basally adnate to each other, always occur on the adaxial (posticus) side of the main axis and the other two free lobes occur abaxially (anticus), forming a so-called two-lipped calyx (Wood and Scotland 2003). The corolla lobes are alternate the calyx lobes. The stamens are unilaterally displaced and adnate to the corolla in the lower portion to form a filament curtain (Manktelow 2000). In the early stage of flower development, the fila-

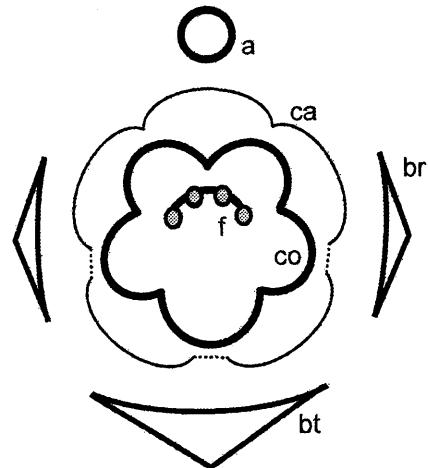


Fig. 3. Schematic diagram of flower parts in *Strobilanthes*, showing relative position of parts to inflorescence axis. a = inflorescence axis. br = bracteole. bt = bract. ca = calyx. co = corolla. f = filament curtain.

ment curtain is always on the posticus side and the curvature of the corolla is indistinct. Later, the corolla more or less bends apically, generally posteriorly, so that the filament curtain occupies a lower position in the corolla tube. In *S. lanyuensis*, however, the corolla bends anteriorly to place the filament curtain above the corolla tube.

Another peculiar floral characteristic in *Strobilanthes* is the twisting of the corolla tube together with the filament curtain, which has been referred as resupination. Since Bremekamp (1944), this character has been recorded to be an important taxonomic character, but difficult to observe in herbarium specimens. Because of the difficulty, Manktelow (2000) avoided using the term 'resupinate' and described the corolla by its shape when fully opened. It is difficult to trace the twist, which occurs at the base of the corolla tube, in pressed herbarium specimens. Since the original position of the filament curtain and the accompanying posticus (upper) corolla lobes are always adaxial, the degree of twisting, or resupination, is easily recognized by the relative position of the filament curtain and the posticus corolla lobes to the inflorescence axis, at least in living specimens, and even in photos. Although Bremekamp (1944) described the corolla of the *Parachampionella* group as not resupinate, it is frequently twisted in the group, according to our field observations. In *S. lanyuensis*, however, we have not seen a twisted corolla.

Manktelow (2000, in appendix 1) described the general trends in the flowers of *Strobilanthes*. Nototribic or sternotribic flowers are seemingly dependent on the degree of torsion in the corolla tube. More or less vertical flowers with a slightly bent corolla tube are nototribic, torsion only 90°, whereas horizontal flowers with a sharply bent corolla tube are sternotribic, torsion 180°. Bennett and Scotland (2003) commented that the filaments are adnate to the

upper surface of the corolla in non-resupinate corollas, and to the lower surface when resupinate. Their observations, however, may be incorrect. The torsion appears to occur mostly in the vertical plane. The relative position of the stamens, nototribic or sternotribic, is independent on the torsion but dependent on the direction of curvature of the corolla tube as explained above.

Stamens: The didynamous filaments in *Strobilanthes* usually consist of longer outer pair and a shorter inner pair. The relative length of the outer and inner pairs varies between species (Bennett and Scotland 2003). In *S. lanyuensis* the free part of the inner pair of filaments appears nearly as long as the outer, while the inner is much shorter than the outer in the other species of the *Parachampionella* group.

Calyx: The calyx of the *Parachampionella* group, including *S. lanyuensis*, is characteristically 2-lipped. In flower the basal fusion of the upper (posticus) three lobes is not distinct but in fruit, when the calyx becomes nearly three times larger than in the flowering stage, the fusion is nearly 1/3 of the entire length and very distinct. The calyx of *S. flexicaulis* usually lacks glandular hairs at flowering time but is frequently covered with glandular hairs in fruit. We observed the development of the glandular hairs during the maturation of the fruits and calyx. Glandular hairs on the calyx have not been found in *S. lanyuensis*.

Distinction of *Strobilanthes lanyuensis*

Consequently, *S. lanyuensis* is clearly distinguished from the other members of the *Parachampionella* group (Table 2). The nototribic staminal deployment is perhaps the most noteworthy feature in *S. lanyuensis*. Field observations of pollinators are necessary to verify its significance. The Lanyu plants were first reported as *S. formosana* by Liu et al. (1955). The Flora of Taiwan (Hsieh and Huang 1978, 1998) lists only *Stro-*

Table 2. Differences of *Strobilanthes lanyuensis* and the other species of the *Parachampionella* group

Characters	<i>S. lanyuensis</i>	<i>S. flexicaulis</i>	<i>S. rankanensis</i>	<i>S. tashiroi</i>	<i>S. perplexa</i>
Pollen grain	ellipsoid	ellipsoid	globose	globose	globose
Corolla twist	non-twisted	twisted	twisted	twisted	twisted
Corolla color	white	light purple~purple	light purple~purple	light purple~purple	blue
Staminal deployment	nototribic	sternotribic	sternotribic	sternotribic	sternotribic
Filament (free part), length ratio outer/inner	ca. 1.4	ca. 2.25	ca. 2.25	ca. 2.25	ca. 2.0
Calyx fusion of upper three teeth (of the total length)	<40%	>40%	>40%	>40%	>40%
Glandular hairs on mature calyx	rarely present	frequently present	absent	absent	absent
Distribution	Orchid Island, Taiwan	Mainland Taiwan; Ryukyu Islands, Japan	Mainland Taiwan	Ryukyu Islands, Japan	Sumatra; Cocos Keeling Islands

bilanthes (*Parachampionella*) *flexicaulis* as occurring on Lanyu, while Hatusima placed his Lanyu specimens in *S. glandulifera* and *S. tashiroi* (Hatusima 1970). However, all the specimens we have seen of *Strobilanthes* from Lanyu agree with *Strobilanthes lanyuensis*.

Taxonomic treatment

***Strobilanthes lanyuensis* Seok, C. F. Hsieh & J. Murata, sp. nov.**

Strobilanthes flexicaulis affinis, a qua corolla albo, non-tortili, staminibus nototribici differt.

Typus: Taiwan, Taitung Hsien, Lanyu Hsiang, Hongtou Village: besides Tienchi,

22 Mar. 2002, D. I. Seok and T. H. Hsieh 0203007 (holotypus TI, isotypus TAI).

[Figs. 4, 5]

Subshrubs, to 1.5 m tall. Stems quadangular, retrorsely pubescent. Leaves opposite, chartaceous; petiole 5–50 mm long, appressed pubescent; blade very variable in form and size, oblong-lanceolate, oblong-ovate or ovate, 3–20 cm long, 2–8 cm wide, base acute or cuneate, attenuate into petiole, apex acute to acuminate, mucronate-serrate, glabrous on both surfaces, nerves rarely sparsely pubescent on lower surface, with many cystoliths, midrib and 3–5 pairs of lateral nerves raised on lower surface. Flowers January to March. Inflorescences

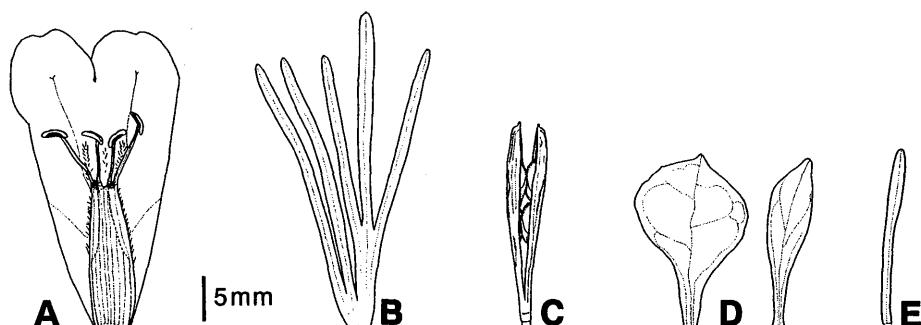


Fig. 4. Floral parts of *Strobilanthes lanyuensis* (Seok & Hsieh 0203007). A: upper corolla lobes with filament curtain. B: calyx at fruiting stage. C: fruit. D: bracts. E: bracteole.

terminal and in upper leaf axils, racemose; axis quadrangular, glabrous or glandular pubescent, usually 5–10 cm long, nodes 2–8.

Bracts obovate, narrowly oblong or linear-oblanceolate, obtuse, 1–2.5 cm long, with many cystoliths, lower surface glabrous or

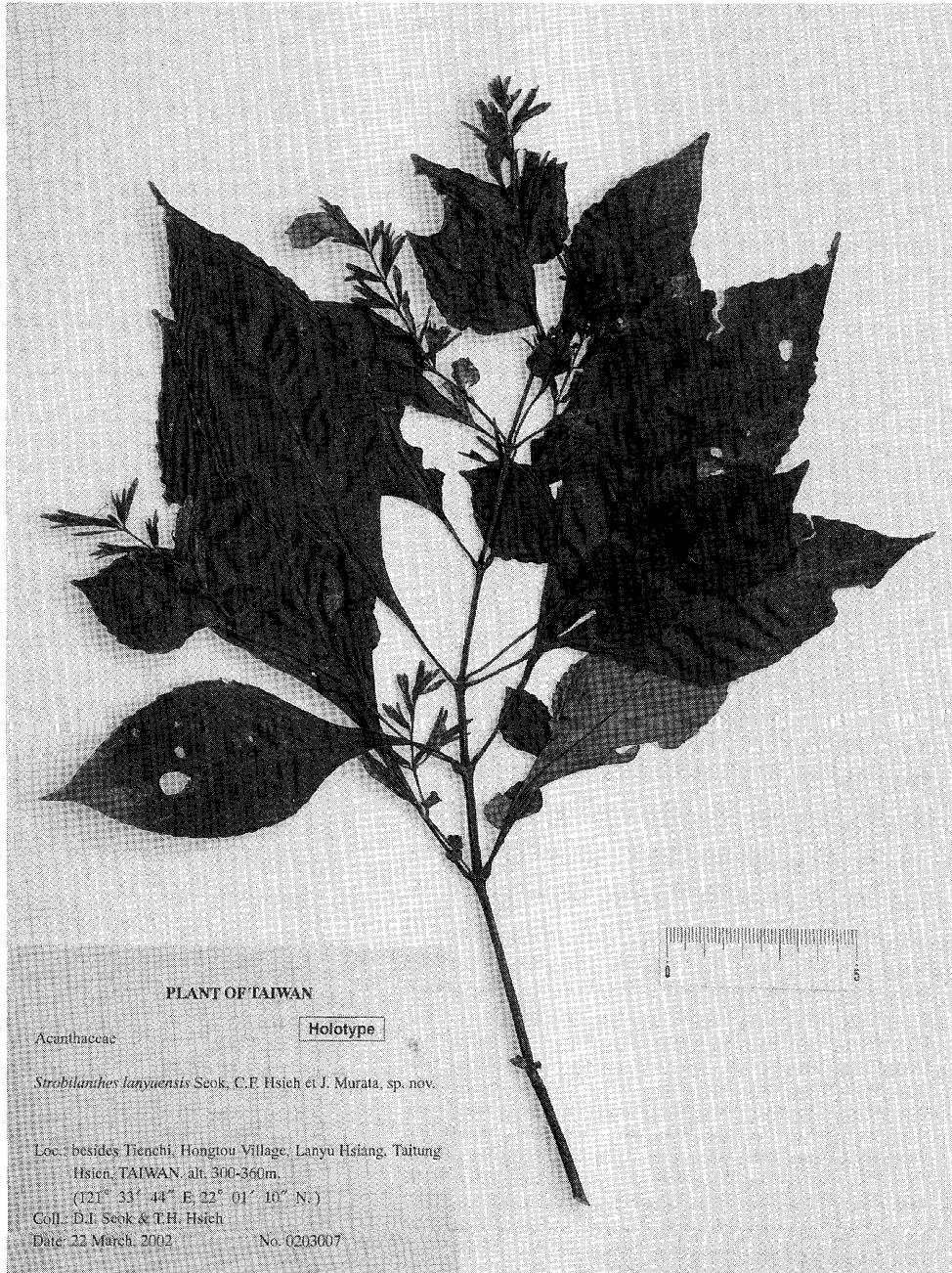


Fig. 5. Holotype of *Strobilanthes lanyuensis* (Seok & Hsieh 0203007, TI).

sparserly pubescent, each subtending a single flower; pedicel 0.5–3 mm long. Bracteoles 2, linear, acute, 10–20 mm long, glabrous or sparserly pubescent. Calyx 13–24 mm long in flower, to 40 mm long in fruit, upper lip 3-lobed to 2/3 its length, lower lip bilobed nearly to base, lobes linear, acute, glabrous, pubescent or margin rarely glandular pilose, with many cystoliths. Corolla not resupinate, tubular-funnelform, glabrous, white, 4 cm long, abaxially curved apically. Stamens 4, didynamous, included, longer pair ca. 2.5 cm long, shorter pair ca. 2 cm long; filaments densely pilose, adnate to adaxial surface of lower 2/3 of corolla tube; anthers oblong, ca. 2 mm long, locules 2, subequal, parallel. Pollen grains ellipsoid, 3-colporate, longitudinal ribs with ladder-like reticulum. Style filiform, ca. 25 mm long, very unequally bilobed. Capsule cylindrical-fusiform, ca. 17 mm long, glabrous. Seeds 4 per capsule, ellipsoid, ca. 3 mm long, densely puberulent.

Specimens examined: Taiwan, Lanyu Island: Tienchi, Hongtou Village (121°33'44"E, 22°01'10"N), alt. 300–360 m., 22 Mar. 2002, D. I. Seok and T. H. Hsieh 0203007; 16 Feb. 1995, Wen-Pen Leu 2007 & al. (TNM); 22 April. 1997, T. Y. A. Yang and C. H. Chu 07988 (TNM); 24 Mar. 1998, T. Y. A. Yang & C. N. Wang 10109 (TNM); 1 April. 1985, S. F. Huang 2710 (TAI); 1 Apr. 1988, T. Y. A. Yang 01497 (TAI). Hongtoushan, Yehyu Village, 24 April. 1997, T. Y. A. Yang & al. 08138 (TNM).

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昔 東姫^a、謝 長富^b、邑田 仁^a：台湾産イセハナビ属（キツネノマゴ科）の1新種とその花形態について

日本・台湾産のイセハナビ属の分類学的再検討の過程で、台湾の蘭嶼から新種を発見したので *Strobilanthes lanyuensis* Soek, C. F. Hsieh & J. Murata と命名・記載する。分子系統解析の結果 *S. lanyuensis* は、台湾産の *S. flexicaulis* Hayata (アリサンアイ), *S. rankanensis* (Hayata) Bremek, 琉球産の *S. tashiroi* (Hayata) Bremek (オキナワスズムシソウ) などとごく近縁な1群 (*Parachampionella* group) をなすことが示された。*S. lanyuensis* はこれら近縁種と外部形態的にもよく似ており、萼片のうち向軸側の3片の基部が合着することは共通しているが、花冠が白く、ねじれず、雄蕊群が花筒の上側に位置する点で明らかに異なる。イセハナビ属における花冠のねじれは、雄蕊の基部が花筒の向軸側に合着して形成する filament

curtain が開花時に花序軸からどれくらい変位しているかでその程度を判断することができる。従来、雄蕊群が花筒の上側にあるか下側にあるかは花冠のねじれと関連づけられてきたが、*S. lanyuensis* で観察した結果、花冠のねじれではなく花冠の曲がりと関連していることが明らかとなった。すなわち、花冠はその上部で片側に曲がるが、filament curtain 側に曲がれば雄蕊群は花冠の下側に位置し、filament curtain と反対側に曲がれば雄蕊群は花冠の上側に位置することになる。このことは送粉者のどこに花粉がつかを決める重要な形質と考えられる。花冠のねじれの程度は同一個体内の花でも一定しておらず、主に水平面内での開口部の位置づけに役立っていると考えられる。

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